

Appl. No.: 10/005,429
Amdt. dated March 25, 2005
Reply to Office Action of December 29, 2004

Amendments to the Claims:

Claims 1-61 (Cancelled)

62. (Currently amended) A method for altering the disulfide status of storage proteins in a plant or part thereof, said method comprising transforming said plant with a first nucleotide construct comprising a first nucleotide sequence and a second nucleotide construct comprising a second nucleotide sequence,

wherein said first nucleotide sequence encodes an NADPH-thioredoxin reductase, said second nucleotide sequence encodes a thioredoxin *h*, and said first nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO: 24;
- b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 25;
- c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequence set forth in SEQ ID NO: 24, wherein said nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity;
- d) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO: 24, wherein said nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity, and wherein said stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC; and
- e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d);

wherein said disulfide status of said storage proteins is ~~altered~~ decreased in said plant or part thereof.

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63. (Previously presented) The method of claim 62, wherein said second nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO: 13;
- b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 14;
- c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequences set forth in SEQ ID NO: 13, wherein said nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity;
- d) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO: 13, wherein said nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity, and wherein said stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC; and
- e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d).

64. (Previously presented) The method of claim 62, wherein said part is seed or grain.

65. (Previously presented) The method of claim 62, wherein at least one of said first and said second nucleotide constructs further comprises an operably linked promoter that drives expression in a plant cell.

66. (Previously presented) The method of claim 65, wherein said promoter is selected from the group consisting of seed-preferred, constitutive, chemically regulated, tissue-preferred, and developmentally regulated promoters.

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67. (Previously presented) A method for improving the digestibility of grain, said method comprising transforming a plant with a first nucleotide construct comprising a first promoter that drives expression in a plant cell operably linked to a first nucleotide sequence and a second nucleotide construct comprising a second promoter that drives expression in a plant cell operably linked to a second nucleotide sequence,

wherein said first nucleotide sequence encodes an NADPH-thioredoxin reductase, said second nucleotide sequence encodes a thioredoxin *h*, and said first nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO: 24;
 - b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 25;
 - c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequence set forth in SEQ ID NO: 24, wherein said nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity;
 - d) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO: 24, wherein said nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity, and wherein said stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC; and
 - e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d);
- wherein said digestibility of said grain is increased when consumed by an animal.

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68. (Previously presented) The method of claim 67, wherein said second nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO: 13;
- b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 14;
- c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequences set forth in SEQ ID NO: 13, wherein said nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity;
- d) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO: 13, wherein said nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity, and wherein said stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC; and
- e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d).

69. (Previously presented) The method of claim 67, wherein at least one of said first and said second promoters is selected from the group consisting of seed-preferred, constitutive, chemically regulated, tissue-preferred, and developmentally regulated promoters.

70. (Previously presented) The method of claim 67, wherein said animal is a ruminant animal or a monogastric animal.

71. (Previously presented) The method of claim 67, wherein the grain is a mature maize kernel.

72. (Previously presented) The method of claim 67, wherein the grain is a kernel that is part of a whole corn plant harvested for silage.

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73. (Previously presented) A method for improving grain for processing, said method comprising transforming a plant with a first nucleotide construct comprising a first promoter that drives expression in a plant cell operably linked to a first nucleotide sequence and a second nucleotide construct comprising a second promoter that drives expression in a plant cell operably linked to a second nucleotide sequence,

wherein said first nucleotide sequence encodes an NADPH-thioredoxin reductase, said second nucleotide sequence encodes a thioredoxin *h*, and said first nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO: 24;
- b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 25;
- c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequence set forth in SEQ ID NO: 24, wherein said nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity;
- d) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO: 24, wherein said nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity, and wherein said stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC; and
- e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d);

wherein said grain is improved for processing.

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74. (Previously presented) The method of claim 73, wherein said second nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO: 13;
- b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 14;
- c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequences set forth in SEQ ID NO: 13, wherein said nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity;
- d) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO: 13, wherein said nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity, and wherein said stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC; and
- e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d).

75. (Previously presented) The method of claim 73, wherein at least one of said first and said second promoters is selected from the group consisting of seed-preferred, constitutive, chemically regulated, tissue-preferred, and developmentally regulated promoters.

76. (Previously presented) The method of claim 73, wherein the processing is wet milling and the improvement is increased starch extractability, decreased need for chemical reducing agents, or improved digestibility of wet milling by-products.

77. (Previously presented) The method of claim 73, wherein the processing is grinding and the improvement is a higher degree of particle size reduction or energy savings.

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78. (Previously presented) The method of claim 73, wherein the processing is steam flaking and the improvement is a larger improvement in digestibility enhancement by steam flaking or energy savings.

79. (Previously presented) The method of claim 73, wherein the processing is dry grind ethanol production and the improvement is increased fermentable starch or increased digestibility of fermentation by-products.

Claims 80-86 (Cancelled)

87. (Currently amended) A transformed plant comprising stably incorporated in its genome a first nucleotide construct comprising a first promoter that drives expression in a plant cell operably linked to a first nucleotide sequence and a second nucleotide construct comprising a second promoter that drives expression in a plant cell operably linked to a second nucleotide sequence,

wherein said first nucleotide sequence encodes an NADPH-thioredoxin reductase, said second nucleotide sequence encodes a thioredoxin *h*, and said first nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO: 24;
- b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 25;
- c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequence set forth in SEQ ID NO: 24, wherein said nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity;
- d) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO: 24, wherein said nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity, and wherein said stringent conditions comprise hybridization at 37°C in a solution comprising in 50%

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formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC; and

e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d)[[:]].

88. (Previously presented) The plant of claim 87, wherein said second nucleotide sequence is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 13;

b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 14;

c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequences set forth in SEQ ID NO: 13, wherein said nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity;

d) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO: 13, wherein said nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity, and wherein said stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC; and

e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d).

89. (Previously presented) The plant of claim 87, wherein said promoter is selected from the group consisting of seed-preferred, constitutive, chemically regulated, tissue-preferred, and developmentally regulated promoters.

90. (Previously presented) The plant of claim 87, wherein said plant is a monocot.

91. (Previously presented) The plant of claim 90, wherein said monocot is selected from the group consisting of maize, wheat, rice, sorghum, barley, millet and rye.

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92. (Previously presented) The plant of claim 87, wherein said plant is a dicot.

93. (Previously presented) The plant of claim 92, wherein said dicot is selected from the group consisting of soybean, *Brassica sp.*, alfalfa, safflower, sunflower, cotton, peanut and potato.

94. (Previously presented) Transformed seed of the plant of claim 87.